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Orchard calcium and fungicide treatments mitigate effects of delayed postharvest fungicide applications for control of postharvest decay of pear fruit

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ABSTRACT

Postharvest decay of pear fruit is commonly initiated at wounds that occur during harvest and handling, yet pears may be held in cold storage for considerable lengths of time before fungicides are applied to prevent postharvest decay. The efficacy of postharvest fungicide treatments declines as the time between harvest and fungicide treatment increases. Foliar sprays of calcium chloride applied to 'Bosc' pear trees beginning in mid-July and repeated every two weeks for a total of three applications slowed the increase in decay incidence observed when thiabendazole or fludioxonil treatments were delayed up to eight weeks after harvest. Increased decay incidence due to delayed postharvest fungicide application was also reduced by application of either pyraclostrobin + boscalid, thiophanate-methyl, or trifloxystrobin one week before harvest. Calcium chloride treatments followed by either of the pre-harvest fungicides provided the greatest decay suppression when postharvest fungicide treatments were delayed. Decay control by fludioxonil treatment was superior to that provided by thiabendazole when the fungicides were applied to fruit promptly after harvest, but differences between these two fungicides decreased as the delay in application approached eight weeks after harvest. A program of orchard calcium chloride followed by pre-harvest fungicide treatments can reduce overall decay incidence and mitigate the consequences of delays in postharvest fungicide treatment.

1. Introduction

Infection of pear fruit during long-term storage, caused by any of several fungal pathogens, can result in significant economic losses for pear producers (Kupferman, 1998). Fungicide application to the fruit after harvest is the primary tactic employed to reduce incidence of postharvest decay in pear packinghouses (Eckert and Sommer, 1967).

In recent decades, most pears packed for long-term storage in the United States were treated with a benzimidazole fungicide (benomyl or thiabendazole). More recently, fungicides containing pyrimethanil and fludioxonil were registered for postharvest application to pears in the United States and elsewhere, and have been shown to be effective against decay of pome fruits (Errampalli, 2004; Vostermans et al., 2005; Xiao and Boal, 2009a). However, the efficacy of these fungicides as well as those of the benzimidazole class is dependent on prompt application. Since most decay in pear fruit is initiated at wounds that occur during harvest and subsequent handling (Spotts et al., 1998), postharvest fungicide treatments are most effective when applied as soon as possible after harvest (Sugar and Basile, 2008).

Pears are harvested during a relatively narrow range of fruit maturity, followed by prompt cooling to remove field heat (Hansen and Mellenthin, 1979). Among the varying methods of postharvest handling employed by commercial operators, opportunities for application of decay control treatments typically occur (1) before fruit are placed in long-term storage, either as high-volume recirculating "drenches" while the fruit are in field bins or as in-line spray treatments during pre-storage sorting and sizing, and (2) as in-line spray treatments immediately before fruit are packed into the boxes in which they are marketed. Many commercial pear operations store pears for extended periods in field bins because the large volume of pears harvested in the maturity period may require several months for sorting and packing to be completed, and because of uncertainty regarding the type of packaging that will be needed to fill specific market demands at the time of sale (Kupferman, personal communication). Pears are often stored in field bins without postharvest fungicide treatment, receiving decay control treatment only as an in-line spray before final packing. Reasons for avoiding drench applications include minimizing risk of inoculating fruit with pathogen spores, especially those of *Penicillium expansum*, that may accumulate in drench solutions (Fidler et al., 1973). Increased incidence of decay caused by *P. expansum* and by *Mucor piriformis* have been associated with pre-storage drenching in field bins (Sanderson et al., 1998; Xiao et al., 2004).

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Treatments applied to fruit in the orchard can affect incidence of decay during long-term storage. Calcium chloride sprays during the growing season enhanced fruit resistance to decay and contributed to integrated pear decay management programs (Sugar et al., 1992, 1994, 2003). Applications prior to harvest of the fungicides benomyl (Ben-Arie and Guelfat-Reich, 1973), cyprodinil (Sholberg et al., 2003), boscalid + pyraclostrobin (Xiao and Boal, 2009b), and ziram (Sugar et al., 2003; Xiao and Boal, 2009b) have been shown to be beneficial in reducing incidence of decay caused by various fungi in pome fruit. However, to our knowledge the impacts of calcium chloride and pre-harvest fungicide treatments on the efficacy of subsequent postharvest fungicide treatments have not been explored previously.

The objective of this study was to determine if summer calcium chloride sprays, fungicide treatments applied one week before harvest, or a sequential treatment program of calcium chloride followed by fungicide treatment can mitigate the decrease in effectiveness of postharvest fungicides due to delay in treatment application.

2. Materials and methods

2.1. Orchard treatments

Orchard experiments were conducted in 2008 and 2009 in a 0.4 ha block of 'Bosc' pear trees planted in 1959 at a spacing of 6 m × 3 m at the Southern Oregon Research and Extension Center near Medford. Calcium chloride was applied to 16 groups of two trees each arrayed through the orchard in a randomized complete block design with four replicate blocks. Calcium chloride was applied at the rate of 11.0 g L^{-1} in mid-July and repeated every two weeks for a total of three applications per season (Sugar et al., 1991). The treatments were applied using a powered handgun sprayer set to deliver 300 L ha^{-1} with solutions applied to run-off. Another set of 16 groups of two trees each were not treated with calcium chloride.

Within each set receiving calcium chloride or no calcium, four groups of two trees each were treated one week before harvest with one of the following fungicides: pyraclostrobin + boscalid [as Pristine® (BASF) at 0.5 g L^{-1}], thiophanate-methyl [as Topsin® M (United Phosphorus) at 0.6 g L^{-1}], trifloxystrobin [as Flint® (Bayer CropScience) at 0.1 g L^{-1}], or no pre-harvest fungicide treatment. Fungicide treatments were applied by powered handgun sprayer as described above.

2.2. Postharvest treatments

At commercial maturity in mid-September of each year, 420 fruit were harvested from each replicate two-tree unit and brought to the laboratory. Each fruit was wounded in three locations with a finishing nail (2 mm diameter × 3 mm depth). Naturally occurring decay fungi present on the fruit surface served as inoculum for subsequent decay development. All wounded fruit were stored in fiberboard boxes in air at −0.5 °C.

On the day following wounding (herein referred to as 0 weeks after harvest), 20 fruit from each orchard replicate were treated with the fungicide fludioxonil (as Scholar® 230SC (Syngenta Crop Protection) at 1.25 mL L^{-1}). An additional set of 20 wounded fruit from each orchard replicate were treated with thiabendazole (as Mertect® 340F (Syngenta Crop Protection) at 1.25 mL L^{-1}), and a third set of 20 wounded fruit from each orchard replicate were treated with water. Postharvest treatments were applied by spraying the fruit while traveling across a series of rotating brushes, simulating a common packinghouse treatment method. All equipment was thoroughly cleaned with water between fungicide treatments. After treatment, each 20-fruit unit was placed in a perforated polyethylene bag. Bags of fruit were grouped in fiberboard boxes and stored in air at −0.5 °C.

Table 1
Summary of analysis of variance for postharvest decay incidence at artificial wounds in 'Bosc' pear fruit that received postharvest treatment with either water, thiabendazole, or fludioxonil at various lengths of time after harvest.

Source of variation	*df*	Mean square	*Pr* > *F*
Postharvest treatment (A)[a]	2	0.061	<0.001
Postharvest treatment timing (B)	6	0.036	<0.001
Year (C)	1	0.004	0.091
A × B	12	0.002	0.218
A × C	2	0.010	0.006
B × C	6	0.001	0.495

[a] Water, thiabendazole (Mertect® 340F, 1.25 mL L^{-1}), or fludioxonil (Scholar® 230SC, 1.25 mL L^{-1}) were applied as line-sprays either 0, 1, 2, 3, 4, 6, or 8 weeks after harvest and wounding in each of two years (2008 and 2009). No fungicide or calcium treatments were applied in the orchard. Fruit were maintained at −0.5 °C after harvest and decay was evaluated after 14 weeks of storage.

At 1, 2, 3, 4, 6, and 8 weeks after harvest, additional sets of 20 fruit wounded at harvest were removed from storage for each orchard replicate and treated with fludioxonil, thiabendazole, or water as described above. At 14 weeks after harvest, all fruit were removed from storage and incidence of decay at wounds was evaluated. Decay lesions were identified visually based on the authors' experience with pear decay. Where pathogen identity was uncertain due to the similar appearance of lesions caused by *Cladosporium herbarum*, *Alternaria* sp., and *Phialophora malorum* (Jones and Aldwinkle, 1990), a subset of fruit (n = 60) were surface-sterilized for 5 min in 0.5% sodium hypochlorite, then tissue from the interior of lesions was plated onto acidified potato dextrose agar (APDA) and pathogens were identified visually after colony development and sporulation. Pathogens were also isolated from a subset of lesions (n = 60) identified as being caused by *P. expansum* each year. Conidia produced by each isolate of *P. expansum* were then spread as lawns on APDA plates. Filter paper disks impregnated with either a solution of thiabendazole at 569 mg L^{-1} or fludioxonil at 200 mg L^{-1} were then placed on each plate. After 48 h incubation at 20 °C, the presence or absence of a zone of inhibition around the disk was noted, indicating whether the isolate was tolerant to each fungicide at that dosage.

2.3. Statistical analysis

The incidence of infection at wounds was calculated for each replicate from each of the factorial treatment combinations as the number of wounds infected as a proportion of the total number of wounds (n = 60). Analysis of variance for main factors and interactions in a factorial design and comparison of slopes of regression lines were carried out on the incidence value data set using Statistix v. 9 software (Analytical Software, Tallahassee, FL). Decay incidence was plotted as a function of the number of weeks after harvest that postharvest fungicides were applied for each of the orchard calcium–fungicide combinations, and regression lines were determined.

3. Results and discussion

Incidence of decay increased with delay in application of postharvest fungicide treatments both with thiabendazole and with fludioxonil (Table 1, Fig. 1). Decay control provided by fludioxonil was significantly (P < 0.05) greater than that provided by thiabendazole when applied promptly after harvest, but as application was delayed up to eight weeks after harvest the incidence of decay in fruit treated with fludioxonil was increasingly similar to that found in fruit treated with thiabendazole. Incidence of decay

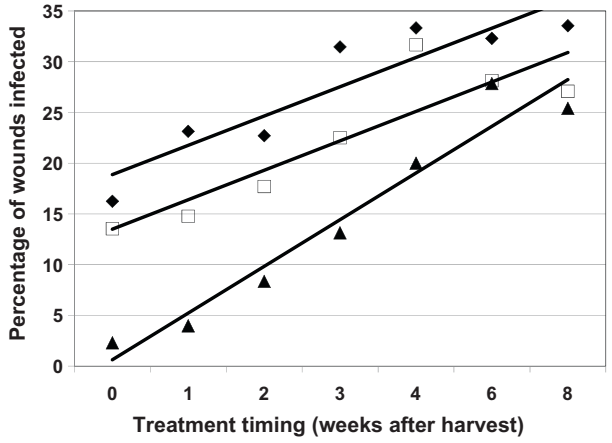

Fig. 1. Effect of delay in application of postharvest fungicides on incidence of decay in 'Bosc' pears in the absence of orchard-based decay control treatments. Fruit were artificially wounded at harvest and stored at −0.5 °C, then treated 0, 1, 2, 3, 4, 6, or 8 weeks after harvest with either water (♦), thiabendazole (□), or fludioxonil (▲) as a line-spray. Regression equations: water: $y = 2.89x + 16.0$, $R^2 = 0.84$; TBZ: $y = 2.90x + 10.6$, $R^2 = 0.79$; FLU: $y = 4.60x - 4.0$, $R^2 = 0.94$.

also increased with delay in postharvest treatment application in the water control (Fig. 1). This may reflect inoculum dilution as a "washing" effect, which would become less of a factor as infections were initiated over time.

Both summer calcium treatments and orchard fungicide treatments applied one week before harvest were significant ($P < 0.05$) factors in reducing postharvest decay incidence at wounds (Tables 2 and 3). Interactions between calcium treatments and pre-harvest fungicide treatments, and between each of these factors and the timing of postharvest fungicide application were significant, both with thiabendazole and with fludioxonil as postharvest treatments (Tables 2 and 3).

Incidence of decay generally increased with delay in application of a postharvest fungicide when either only calcium chloride or only a pre-harvest fungicide was applied in the orchard, but the incidence of decay after eight weeks was still lower for these treatments than for fruit that received no pre-harvest treatment

Table 2
Summary of analysis of variance for postharvest decay incidence at artificial wounds in 'Bosc' pear fruit as affected by the timing of thiabendazole postharvest treatments and by pre-harvest foliar sprays.

Source of variation	*df*	Mean square	*Pr* > *F*
Calcium treatment (A)[a]	1	0.611	<0.001
Pre-harvest fungicide (B)[b]	3	0.171	<0.001
Postharvest fungicide timing (C)[c]	6	0.020	<0.001
Year (D)	1	0.009	0.170
A × B	3	0.029	<0.001
A × C	6	0.011	0.048
A × D	1	0.004	0.367
B × C	18	0.012	0.002
B × D	3	0.010	0.098
C × D	6	0.007	0.215
A × B × C	18	0.005	0.363
A × B × D	3	0.021	0.005
A × C × D	6	0.007	0.173
B × C × D	18	0.007	0.087

[a] Orchard trees received either three foliar sprays of calcium chloride (11.0 g L^{-1}) or no calcium.

[b] Either pyraclostrobin + boscalid (Pristine®, 0.5 g L^{-1}), thiophanate-methyl (Topsin® M, 0.6 g L^{-1}), trifloxystrobin (Flint®, 0.1 g L^{-1}), or no fungicide were applied one week pre-harvest.

[c] Thiabendazole (Mertect® 340F) was applied at 1.25 mL L^{-1} either 0, 1, 2, 3, 4, 6, or 8 weeks after harvest and wounding in each of two years (2008 and 2009). Fruit were maintained at −0.5 °C after harvest and decay was evaluated after 14 weeks of storage.

Table 3
Summary of analysis of variance for postharvest decay incidence at artificial wounds in 'Bosc' pear fruit as affected by the timing of fludioxonil postharvest treatments and by pre-harvest foliar sprays.

Source of variation	*df*	Mean square	*Pr* > *F*
Calcium treatment (A)[a]	1	0.266	<0.001
Pre-harvest fungicide (B)[b]	3	0.126	<0.001
Postharvest fungicide timing (C)[c]	6	0.061	<0.001
Year (D)	1	0.001	0.671
A × B	3	0.048	<0.001
A × C	6	0.016	<0.001
A × D	1	0.001	0.665
B × C	18	0.009	<0.001
B × D	3	0.003	0.332
C × D	6	0.001	0.835
A × B × C	18	0.004	0.126
A × B × D	3	0.000	0.964
A × C × D	6	0.002	0.531
B × C × D	18	0.002	0.703

[a] Orchard trees received either three foliar sprays of calcium chloride (11.0 g L^{-1}) or no calcium.

[b] Either pyraclostrobin + boscalid (Pristine®, 0.5 g L^{-1}), thiophanate-methyl (Topsin® M, 0.6 g L^{-1}), trifloxystrobin (Flint®, 0.1 g L^{-1}), or no fungicide were applied one week pre-harvest.

[c] Fludioxonil (Scholar® 230SC) was applied at 1.25 mL L^{-1} either 0, 1, 2, 3, 4, 6, or 8 weeks after harvest and wounding in each of two years (2008 and 2009). Fruit were maintained at −0.5 °C after harvest and decay was evaluated after 14 weeks of storage.

(Figs. 2 and 3). The slopes of the regression lines describing the relationship between decay incidence and delay in application of postharvest fungicides were significantly different ($P < 0.05$) where orchard calcium chloride, pre-harvest fungicide, or calcium chloride followed by pre-harvest treatments were applied than where no orchard treatments were applied, for all of the pre-harvest fungicides tested (data not shown). The least increase in incidence of decay as application of postharvest fungicide was delayed from 0 to 8 weeks after harvest resulted from the combined treatment of summer calcium chloride followed by fungicide applied one week before harvest (Figs. 2 and 3).

Foliar sprays of calcium chloride solutions to pear trees can result in increased concentrations of calcium in the fruit tissues, greater firmness retention at harvest, and reduced postharvest decay severity as compared to fruit from untreated trees (Sugar et al., 1994). Conway and Sams (1983) and Conway et al. (1994) showed that calcium ions from exogenous treatments can associate with pectic materials in apple fruit structure, and potentially inhibit pectin-degrading enzymes elaborated by decay fungi.

The three fungicides applied one week before harvest in this study, pyraclostrobin + boscalid, thiophanate-methyl, and trifloxystrobin all reduced the rate of increase in decay incidence with delay in application of postharvest thiabendazole or fludioxonil at the rates applied. When only water was applied as a postharvest treatment, incidence of decay at wounds following any of these pre-harvest fungicides was significantly ($P < 0.05$) less than when no orchard treatment was applied. Incidence of decay following thiophanate-methyl and trifloxystrobin treatments did not differ between these treatments, while incidence of decay following pyraclostrobin + boscalid was significantly less than either thiophanate-methyl or trifloxystrobin (data not shown).

The active ingredients in these fungicides represent three distinct fungicide classes with respect to resistance management: quinone outside inhibitors (pyraclostrobin and trifloxystrobin), methyl benzimidazole carbamates (thiophanate-methyl), and carboximides (boscalid) (FRAC, 2007). The postharvest fungicides used in this study belong to the benzimidazole carbamate class (thiabendazole) and phenylpyrroles (fludioxonil). Since thiophanate-methyl and thiabendazole are in the same fungicide class, their sequential use should be avoided in the interest of resis-

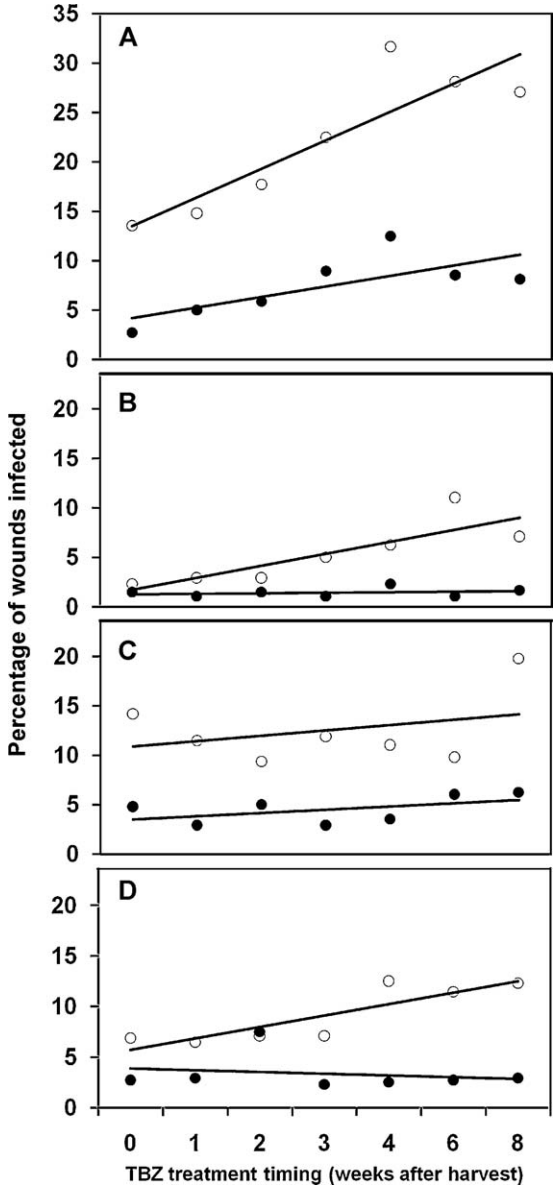

Fig. 2. Effects of orchard treatments on the incidence of postharvest decay in 'Bosc' pears when postharvest treatment with thiabendazole (TBZ) is delayed. Orchard trees received either summer foliar sprays of calcium chloride (●) or no calcium treatment (○), followed by fungicide applied one week pre-harvest. Preharvest fungicides were either (A) none, (B) pyraclostrobin + boscalid, (C) thiophanate-methyl, or (D) trifloxystrobin. Regression equations: no orchard treatment: $y = 2.90x + 10.6$, $R^2 = 0.79$; calcium chloride: $y = 1.07x + 3.1$, $R^2 = 0.53$; pyraclostrobin + boscalid: $y = 1.21x + 0.5$, $R^2 = 0.72$; calcium chloride followed by pyraclostrobin + boscalid: $y = 0.05x + 1.2$, $R^2 = 0.06$; thiophanate-methyl: $y = 0.54x + 10.3$, $R^2 = 0.11$; calcium chloride followed by thiophanate-methyl: $y = 0.33x + 3.2$, $R^2 = 0.26$; trifloxystrobin: $y = 1.13x + 4.6$, $R^2 = 0.76$; calcium chloride followed by trifloxystrobin: $y = -0.17x + 4.0$, $R^2 = 0.04$.

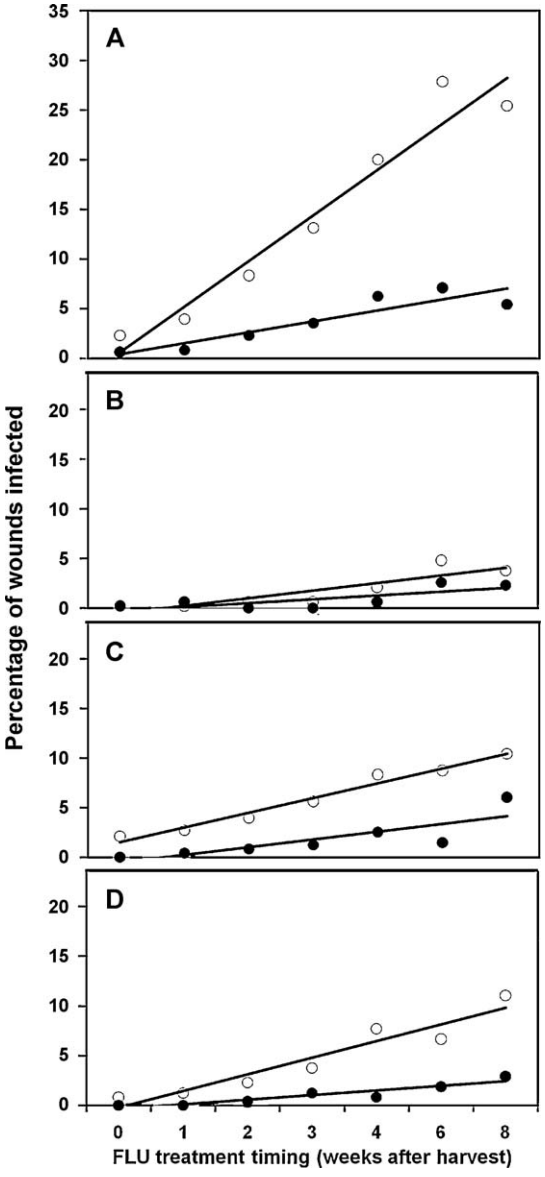

Fig. 3. Effects of orchard treatments on the incidence of postharvest decay in 'Bosc' pears when postharvest treatment with fludioxonil (FLU) is delayed. Orchard trees received either summer foliar sprays of calcium chloride (●) or no calcium treatment (○), followed by fungicide applied one week pre-harvest. Pre-harvest fungicides were either (A) none, (B) pyraclostrobin + boscalid, (C) thiophanate-methyl, or (D) trifloxystrobin. Regression equations: no orchard treatment: $y = 4.60x - 4.0$, $R^2 = 0.94$; calcium chloride: $y = 1.10x - 0.7$, $R^2 = 0.84$; pyraclostrobin + boscalid: $y = 0.76x - 1.3$, $R^2 = 0.78$; calcium chloride followed by pyraclostrobin + boscalid: $y = 0.38x - 0.6$, $R^2 = 0.59$; thiophanate-methyl: $y = 1.48x + 0.1$, $R^2 = 0.97$; calcium chloride followed by thiophanate-methyl: $y = 0.78x - 1.3$, $R^2 = 0.68$; trifloxystrobin: $y = 1.67x - 1.9$, $R^2 = 0.91$; calcium chloride followed by trifloxystrobin: $y = 0.46x - 0.8$, $R^2 = 0.87$.

tance management (Brent and Hollomon, 1998). Although each of these fungicides is registered for use on pear in the United States, the minimum pre-harvest interval for application of trifloxystrobin is 14 d, according to the label for Flint®.

The activity of fungicides applied one week before harvest on the protection of wounds and surrounding fruit tissue after harvest may be due to direct action on fungal inocula present on the fruit surface, fungicide on the fruit surface being mechanically driven into the wound as the wound is created, or general penetration of the fruit cuticle and sub-cuticular tissues. Xiao and Boal (2009b) suggested that effects of pre-harvest treatment with Pristine fungicide (pyraclostrobin + boscalid) were evident in non-wounded apples that had been washed and brushed at packing 5 mo after harvest.

The diminishing efficacy of postharvest fungicide treatments with delay after harvest in controlling postharvest decay confirms our previous experience (Sugar and Basile, 2008). It is likely that mycelial development and tissue penetration had increasingly proceeded beyond the capacity for inhibition by the fungicides used in this study.

In 2008, 83.5% of the total decay was caused by *P. expansum*, 6.8% by *Alternaria* sp., 5.8% by *Botrytis cinerea*, and 2% by *C. herbarum*. In 2009 42.6% of the total decay was caused by *C. herbarum*, 29.6%

by *Alternaria* sp., 20.5% by *P. expansum*, and 3% by *B. cinerea*. In each year a lesser incidence of decay was caused by *Phacidiopycnis washingtonensis*, *Neofabraea* sp., and *Phialophora malorum*. In each year approximately 10% of the isolates of *P. expansum* tested were not inhibited in vitro by thiabendazole at 569 mg L^{-1}, while all isolates were inhibited by fludioxonil. The improved control provided by fludioxonil when applied promptly after harvest relative to the control provided by thiabendazole likely reflects differences in fungicide sensitivity among the pathogens encountered in this study. Thiabendazole is generally ineffective against *Alternaria* spp. (Jones and Aldwinkle, 1990), while fludioxonil has been found to inhibit *Alternaria* sp. isolated from lesions on pear (Sugar, unpublished).

Thiabendazole resistance in *P. expansum* isolates from infected pears has long been known in the Pacific Northwest (Bertrand and Saulie-Carter, 1978). In the present study, fludioxonil was able to inhibit the thiabendazole-tolerant isolates of *P. expansum*, as has been demonstrated previously (Errampalli and Crnko, 2004; Li and Xiao, 2008).

4. Conclusions

Results in this study indicate the value of orchard treatments with calcium chloride and pre-harvest fungicides in mitigating the consequences of delay in application of postharvest fungicides to pear fruit intended for long-term storage. They also emphasize the value of prompt application of postharvest fungicides in management of postharvest decay.

Acknowledgement

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